METAMORPHOSIS OF *POLYPEDATES MACULATUS* (GRAY, 1830): AN ANALYSIS OF CROWDING EFFECT.

P. K. MISHRA & M. C. DASH [1]

Department of Zoology, Larambha College,
Larambha, Sambalpur, Orissa, India

School of Life Sciences, Sambalpur University,
Jyoti Vihar, Burla - 768017, Sambalpur, Orissa, India

ABSTRACT. - Polypedates maculatus *(Gray, 1830) larvae were reared in isolation and at various degrees of crowding under controlled laboratory conditions and at controlled food level sufficient for the highest density. In laboratory populations, mortality rate during the larval period was independent of initial density but the proportion of the population that successfully completed metamorphosis was a negative exponential function of density. The results clearly indicate a threshold size for initiation of metamorphic transformation. The degree of crowding ultimately does not affect this transformation size. The real effect of crowding is nullified by the effect of the time.*

INTRODUCTION

Polypedates maculatus (Gray, 1830), (formerly known as *Rhacophorus maculatus*) an arboreal frog, usually breeds in grassy and shady damp grounds near the water line and in old manure pits. On an average the clutch size is 622 and the hatching percentage is as high as 40.3. Often more than one egg foam has been collected from a single spot during monsoon. Because of this,

1. Address for correspondence.

P. maculatus larvae occur in crowded condition in nature.

Previous studies on amphibian larvae indicate that the crowding has a negative effect on growth rate and body size at metamorphosis (RICHARDS, 1958; ROSE, 1960; LICHT, 1967; BROCKELMAN, 1969; WILBUR, 1972, 1976 and 1977; DE BENEDICTIS, 1974). In *Rana tigerina* tadpoles increasing density causes slower growth, delayed metamorphosis and a reduction in transformation size (DASH & HOTA, 1980). This arises out of a size-specific competition for food (HOTA & DASH, 1981). But natural occurrence of crowding in *R. tigerina* larvae is yet undetermined. Instead, *P. maculatus* larvae perhaps experience crowding in nature. So in the present paper an attempt has been made to analyse the effect of crowding, if any, on the metamorphic process of *P. maculatus* larvae.

MATERIAL AND METHODS

Spawn were collected from the froggery of the School of Life Sciences (Sambalpur) in mid week of June and were brought to the laboratory for hatching under laboratory conditions (Temp. 30°C $\pm$ 3; Relative humidity 48 % maximum and 22 % minimum) with a natural photoperiod. After hatching, the larvae were mixed to create a genetically homogeneous population at the start of the experiment. They were reared in glass aquaria in conditioned tap water (NACE & RICHARDS, 1972). Each population was transferred to a clean container with clear water and fresh food thrice weekly.

After the third day of hatching two experiments were carried out.

(1) In order to determine the normal growth and larval period, twenty larvae were reared alone in three replicates as the control set until complete resorption of the tail.

(2) Populations of six densities (5, 10, 20, 40, 80 and 160) were reared with the same food ration (sufficient for higher density populations) in three replicates in glass aquaria (45 x 30 x 25 cm) with three litres of active space. Populations were examined weekly twice to determine survival. The masses of a sample of 5 individuals were determined ($\pm$ 0.001 g precision) to ascertain the mean growth rates. Metamorphosed individuals (froglets with emergent fore-limbs) were removed from the population to an amphibious condition for completion of the metamorphosis.

A ration of 7 g of mixed diet (DASH & HOTA, 1980) was given as food from the third day after hatching to each experimental population in the proportion 5: 1: 1 (fresh leaves of *Amaranthus tricolor* (L.): boiled egg yolk: minced and cooked goat meat) until the fifteenth day. It was then increased to 10 g in the proportion of 7: 1.5: 1.5 until twenty second day and then the ration was increased proportionately to a total of 15 g until the completion of metamorphosis.

All statistical analyses of the results were done according to SOKAL & ROHLF (1969). Multiple regression analysis was done according to SNEDECOR & COCHRAN (1967).

RESULTS AND DISCUSSION

1. GROWTH AND LARVAL PERIOD

Fig. 1 gives the body mass histogram of *P. maculatus* larvae in the control set. It shows that genetic and environmental differences can be accounted for by the variances in the body mass of the early larval period. Neither the initial size of the larvae nor the size at metamorphosis correlates with the length of larval period. Growth pattern is sigmoid, body mass reaches the maximum before metamorphic climax and then there is a significant decrease in the body mass. This is because resorption of the long tail serves as the nutrient supply to the fasting metamorphosing animals but is not sufficient to cause a growth in the froglet. These data serve as the control for the crowding effect.

2. LABORATORY POPULATION

Survival of amphibian larvae is predicted by a non-linear model with normal error terms (WILBUR, 1976). Thus mortality rates were computed by using the equation:

$$N_t = N_o e^{-mt}$$

where N_t is the number of survivors before metamorphosis, N_o initial density, m mortality rate, t time of metamorphosis. Table I gives the mortality rates and standard error of the mean from the data of three replicates. A visual inspection of the slope suggests that mortality rate is not related to initial density of the population. Results from Bartlett's test (Adjusted

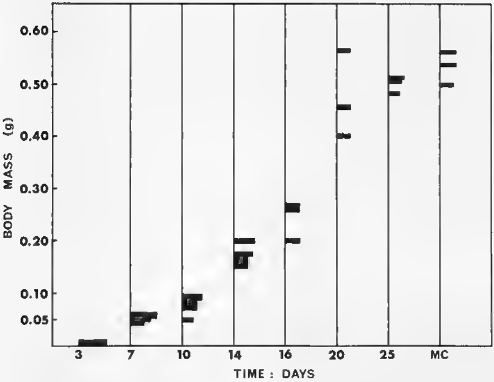

Fig. 1. - Body mass frequency histograms of *Polypedates maculatus* larvae raised in isolation.

Table I. - Mortality rates in laboratory populations of *Polypedates maculatus* larvae.

Initial density	Mortality rate (regression coefficient $\pm$ standard error)
5	0.0113 $\pm$ 0.0071
10	0.0058 $\pm$ 0.0027
20	0.0050 $\pm$ 0.0030
40	0.0056 $\pm$ 0.0009
80	0.0066 $\pm$ 0.0014
160	0.0129 $\pm$ 0.0004

Table II. - Regression analysis of natural logarithm of P_m (the proportion of the population completing metamorphosis) on N_o (the initial density of the population). df: degree of freedom; SS: sum of squares; MS: mean squares; F: ratio of the mean square due to regression to the deviations mean square; P: probability.

Source	df	SS	MS	F	P	Coefficient of determination
Regression	5	21.5619	4.3124	81.9847	0.001	97 %
Residual	12	0.6307	0.0526			
Total	17	22.1926				

$X^2 = 13.3547$, df = 5, $P < 0.005$) showed that variances among replicates at different densities were not homogeneous and it rejected the hypothesis of homogeneity of slopes. This indicates that the mortality rate is independent of crowding.

Regression analysis (Table II) shows that the proportion of the population that metamorphoses is an exponential function of initial density (r^2 = 97 %):

$$P_m = \frac{n_m}{N_o} = e^{-sN_o}$$

which can be fitted by a semilogarithmic model: $\ell n\ P_m = -\ sN_o$. These results indicate that though the mortality rate is independent of density, the absolute mortality is not. The paradox can be better understood by examining the growth rates under crowded conditions (fig. 2).

On days 13, 21 and 25 during the linear phase of growth the masses of all survivors in three replicates of all densities excluding 160 were determined (Table III). A three way factorial analysis of variance was used to examine the main effects of initial density and time. The analysis shows that initial density has a significant effect ($P < 0.001$) on larval growth rate. The sigmoid growth curves (fig. 2) show a distinct right hand shift with the crowding. This suggests crowding inhibits the growth rate of an individual in the population.

Table III. - Analysis of variance of body mass of *Polypedates maculatus* larvae as a function of initial density. df: degree of freedom; SS: sum of squares; MS: mean squares; F: a ratio of two variances as per analysis of variances test; *: P < 0.001; **: P < 0.005.

Density	Time in days	Mean body mass (mg); sample sizes in parentheses		
		Replicates		
		1	2	3
	13	98 (5)	94 (5)	97 (5)
5	21	289.3 (3)	350 (3)	386 (3)
	25	469.3 (3)	474.6 (3)	466.6 (3)
	13	125.8 (10)	172.6 (10)	164.15 (10)
10	21	303 (3)	437.7 (3)	474 (3)
	25	389.6 (3)	465.3 (3)	466.6 (3)
	13	165 (5)	200 (5)	148 (5)
20	21	459 (5)	537.6 (5)	417 (5)
	25	515 (5)	482 (5)	509.8 (5)
	13	104 (5)	91.8 (5)	90.4 (5)
40	21	304 (5)	299.6 (5)	320.2 (5)
	25	410.8 (5)	396.4 (5)	453.8 (5)
	13	83.2 (10)	69.4 (10)	80 (10)
80	21	189.6 (10)	198.8 (10)	198 (10)
	25	260 (10)	252.5 (10)	202.25 (10)

Analysis of variance

Source	df	SS	MS	F	
Density	4	224159.1	56039.775	75.55	*
Replicate	2	4990.5	2495.25	3.36	
Time	2	714823.3	357411.65	481.86	*
Density x Replicate	8	18063.9	2257.98	ns	
Density x Time	8	53632.8	6691.6	9.02	**
Replicate x Time	4	5052.3	1263.07	ns	
Density x Replicate x Time	16	11867.7	741.73		
Total	44	1032489.6			

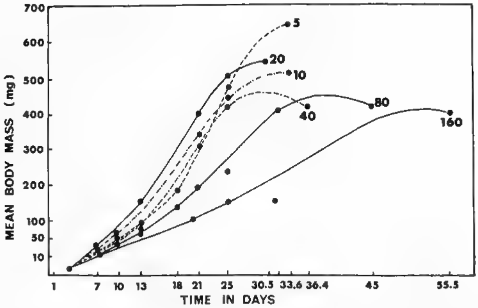

Fig. 2. - Density dependent growth rate of *Polypedates maculatus*. Numbers are individuals per 3 litre aquarium.

Body mass at metamorphosis was analysed by multiple regression analysis with time as the covariate of initial density. The regression is described by the equation $M = 555.02 + (-1.4388)\ N_o + (0.4895)\ t$ ($r^2 = 0.60$; $F = 2.278$; df = 2, 3; P -ns), where M = body mass at metamorphosis, N_o = initial density, t = days until metamorphosis. The regression of body mass at metamorphosis on initial density with time as the covariate is not significant. The regression equation indicates that the negative effect of initial density is nullified by the time of metamorphosis. The curves (fig. 2) also show that the slower growing individuals of higher densities tend to metamorphose at a later date than the rapidly growing individuals of the lower densities. Fig. 3 indicates the effect of initial density on body mass at metamorphosis is insignificant. The lability of the larval period and a more or less constant body mass at metamorphosis at different degrees of crowding very clearly suggests that *P. maculatus* larvae have a specific metamorphic size (threshold size). This confirms the proposition of WILBUR & COLLINS (1973) and DASH & HOTA (1980) that metamorphosis is initiated only after a threshold size is reached. Metamorphic transformation is an "all or none"

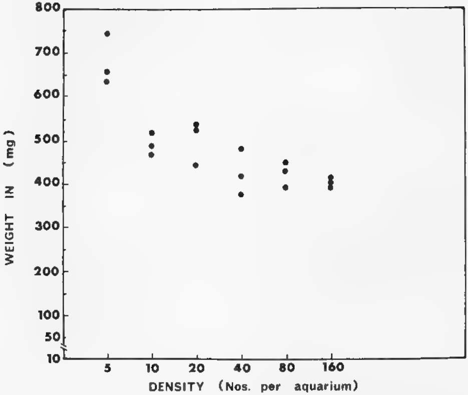

Fig. 3. - Body mass at metamorphosis of *Polypedates maculatus* as a function of initial density of the population.

phenomenon and is initiated after the threshold size.

The size specific competition in *Rana tigerina* (DASH & HOTA, 1980; HOTA & DASH, 1981) had yielded shifts in the time and threshold value of metamorphosis with the degree of crowding. In contrast to this, in this experiment only the time of metamorphosis has shifted without bringing any significant reflection on the threshold value. Competition is the cause and effect of density dependence in anuran larvae (WILBUR, 1976; 1977). The results and its analysis show that the competition of *R. tigerina* and *P. maculatus* are different. In *P. maculatus* the negative modulation of crowding on growth kinetics is compensated by the effect of time to reach the metamorphic climax.

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RESUME

Polypedates maculatus est une grenouille arboricole asiatique qui dépose ses oeufs dans un nid d'écume au bord de l'eau. Les têtards gagnent l'eau après l'éclosion. Chaque ponte comporte en moyenne 622 oeufs dont le pourcentage d'éclosion est de 40,3 %. Il peut y avoir plusieurs pontes autour d'un même point d'eau. Il arrive ainsi fréquemment dans la nature que les têtards de cette espèce soient soumis à de fortes concentrations dans de petites collections d'eau.

Afin d'étudier les effets de cette concentration, des larves de *P. maculatus* ont été élevées au laboratoire isolément et à divers degrés de concentration. La nourriture était fournie en quantité suffisante pour satisfaire à la plus forte densité. On constate, dans les conditions de laboratoire, que le taux de mortalité pendant la période larvaire est indépendant de la densité initiale mais que la proportion de la population qui réussit à se métamorphoser est une fonction exponentielle négative de la densité. La densité initiale a un effet significatif sur la croissance larvaire qui est ralentie par la concentration. Cet effet est compensé par l'allongement de la durée de vie larvaire (retard de la métamorphose) de sorte que la taille à la métamorphose n'est pas modifiée. Les résultats montrent qu'il existe une taille minimale au-dessous de laquelle la métamorphose ne peut commencer.

Chez une espèce comme *Rana tigerina* où il n'est pas prouvé que de fortes concentrations de têtards existent dans les conditions naturelles, on a constaté (DASH & HOTA, 1980) que l'accroissement de leur densité entraînait également un ralentissement de la croissance et un retard de la métamorphose, mais aussi une réduction de la taille à la métamorphose contrairement à *P. maculatus*.

(Résumé rédigé par J.-J. MORERE)

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